



Nitric oxide generation by two *Capsicum annuum* varieties with varying sensitivity to *Phytophthora capsici*

¹REQUENA Maria Emilia, ¹EZZIYANI Mohammed, ²GILABERT Catalina Egea, ³HAMDACHE Ahlem, ³LAMARTI Ahmed and ¹CANDELA Maria Emilia

¹Department of Plant Biology, Faculty of Biology, University of Murcia, Campus de Espinardo, 30100 Espinardo (Murcia), Spain.

²Department of Science and Agrarian Technology. ETS Ingeniería Agronómica. University Polytechnic of Cartagena. Paseo Alfonso XIII, 48. 30203 Cartagena, Spain; ³Faculté des Sciences, Département de Biologie, Equipe de Biotechnologies Végétales. M'hannech II, BP. 2121. 93002 Tétouan (Maroc).

Corresponding author email: mcandela@um.es

ABSTRACT

Objective

To investigate the presence of an NO generation system (NO and NOS) in two pepper varieties showing different degrees of sensitivity to *P. capsici*. In a comparison of resistant and sensitive varieties, we attempted to ascertain the involvement of NO in triggering the defensive reactions and any correlation with the spread of the mould. We discuss the role played by NO in arresting mouldy growth in the resistant variety, and analyse the possible production of NO by NOS in pepper plants.

Methodology and results

The resistant *C. annuum* L. var. Serrano Criollo de Morelos (SCM) and the susceptible var. Americano (AM) were grown in a Fison chamber with a 16 h photoperiod at 25°C and 75–80% RH. *P. capsici* Leon, isolate 17, was maintained in the dark on potato dextrose agar (PDA) medium at 24°C. Ten plants of both varieties were infected when they had five to six true leaves by cutting off the tops of the stems and infecting with plugs of actively growing *P. capsici* mycelium. The control stems were inoculated using only PDA medium without mycelium. To measure the hypersensitive response, the necrotic part of the plants was analysed 3, 6 and 9 days after infection, while penetration of the water mould and the NO–NOS system were evaluated in the intermediate part of the stems (immediately below the necrotic part) 3 and 6 days after infection. Bio-imaging of nitric oxide (NO) *in vivo* was carried out using diaminofluorescein diacetate (DAF-2DA) in conjunction with confocal laser scanning microscopy. Both pepper varieties developed a hypersensitive reaction and generated NO as a defence reaction. However, only SCM was able to overcome the infection by inhibiting the pathogen's growth, while AM succumbed to the disease. NO-induced fluorescence was always higher in SCM than in AM tissues, and was also detected at trace level in decapitated non-infected control plants as a result of wounding. In var. SCM, the highest intensity of NO production (23 pixels) was detected 3 days after infection in the first four sections following the necrosis zone, which corresponded to the mould invasion zone. In AM, extensive necrosis was followed by general invasion of the hyphae, accompanied by minimal NO production.

Conclusion and application of findings

These findings suggest that NO is involved in the resistance reaction against this water mould and that it is induced by the isoenzyme, nitric oxide synthase (NOS), whose activity is qualitatively greater in the tissues of var. SCM. This is the first time that the involvement of NO and NOS has been reported in the defensive signalling reaction between pepper plants and *P. capsici*.

Key words: *Capsicum annuum*, resistance, Nitric oxide, *Phytophthora capsici*

Acknowledgements: This work was partly supported by the project: BFU2004-04707-C02-01 from the Ministry of Science and Technology. CICYT- PGC Plan National I+D.